Aminoterminal propeptide of type III procollagen in the follow-up of patients with abdominal aortic aneurysms

Jari Satta, MD, Kari Haukipuro, MD, PhD, Matti I. Kairaluoma, MD, PhD, and Tatu Juvonen, MD, PhD, *Oulu, Finland*

Purpose: We evaluate here whether serial changes in the concentration of the aminoterminal propeptide of type III procollagen (PIIINP) in serum bear any relationship to the rate of abdominal aortic aneurysm (AAA) expansion and whether serum PIIINP has any predictive value with respect to the rupture event.
Methods: One hundred thirty-nine patients with asymptomatic AAAs were followed-up at intervals of 6 to 12 months by means of a clinical examination, B-mode ultrasound scan, and serum markers of collagen metabolism. Similar laboratory samples were also obtained from 18 patients who had a rupture of the AAA as their primary symptom soon after onset.
Results: The primary correlation between serum PIIINP and AAA diameter was 0.22 ($p = 0.01$), and that between serum PIIINP and the thickness of the thrombus was 0.49 ($p = 0.001$). Toward the end of the follow-up, however, the correlation increased to 0.55 ($p = 0.002$) for serum PIIINP and diameter, but remained at 0.42 ($p = 0.02$) for serum PIIINP and the thickness of the thrombus. Serum PIIINP values were very high among the 18 patients who had ruptured AAAs.
Conclusions: Acceleration of AAA growth is reflected in serum PIIINP, and a marked elevation of serum PIIINP during follow-up of a patient with an AAA may predict an approaching rupture event. (J Vasc Surg 1997;25:909-15.)

Collagen and elastin are the major extracellular matrix proteins responsible for the structural integrity of the aorta, and the interstitial collagen types I and III represent a large proportion of the aortic collagen, with type I predominating. The extensile characteristics, however, are attributed to type III collagen.[1]

Destruction of the elastin, by whatever mechanisms this process may occur, is recognized as a key element in aneurysm formation, its effect being to shift the load produced by the blood pressure on to the collagen, which is assumed to lead to progressive dilatation until rupture occurs.[2] The collagen concentration in abdominal aortic aneurysms (AAAs) has been found in previous investigations to be deficient,[3] unchanged,[4] or increased.[5] Because collagen is continually synthesized throughout life, the collagen content of the aortic wall reflects the net effect of synthesis and degradation.

Changes in total collagen metabolism are now easy to evaluate by measuring concentrations of the circulating procollagen propeptides in serum. The aminoterminal propeptide of type III procollagen (PIIINP)[6] reflects the turnover and the carboxyterminal propeptide of type I procollagen (PICP)[7] reflects the synthesis of the respective collagen. We have recently pointed out an increased turnover of type III collagen in patients with AAAs when compared with patients who have aortoiliac occlusive disease. This increased turnover was established by measuring the levels of PIIINP in peripheral blood. Furthermore, the finding that PIIINP levels increase across the aneurysm sac suggested increased local collagen turnover within the aneurysm.[8]

The purpose of this study was to determine whether serial changes in serum PIIINP (s-PIIINP) bear any relationship to the rate of AAA expansion, and whether s-PIIINP concentration has any predictive value with respect to the rupture event.

PATIENTS AND METHODS

One hundred thirty-nine patients with asymptomatic AAAs were under observation, and 18 pa-

From the Department of Surgery, University of Oulu and Oulu University Hospital.
Reprint requests: Tatu Juvonen, MD, PhD, Assistant Research Professor, Department of Cardiothoracic Surgery, Mount Sinai Medical Center, One Gustave L. Levy Place, Box 1028, New York, NY 10029.

0741-5214/97/$5.00 + 0 24/1/78697

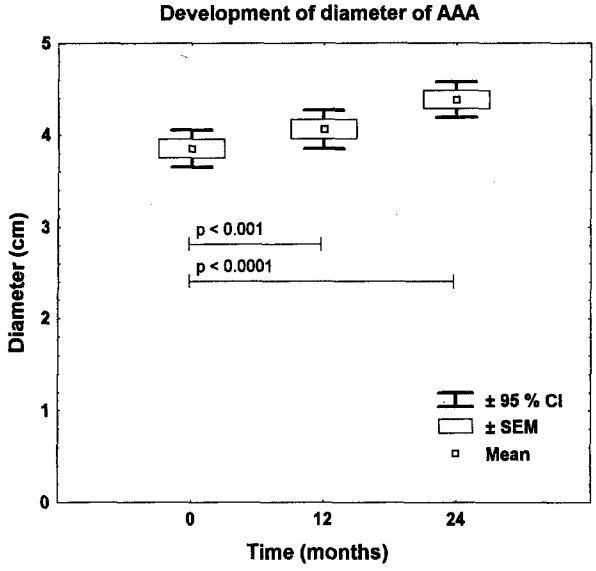

Fig. 1. Diameter of AAA in 55 patients monitored for at least 24 months. $p < 0.0001$ (analysis of variance for repeated measurements). Results of post-hoc evaluation were calculated by Scheffe's method.

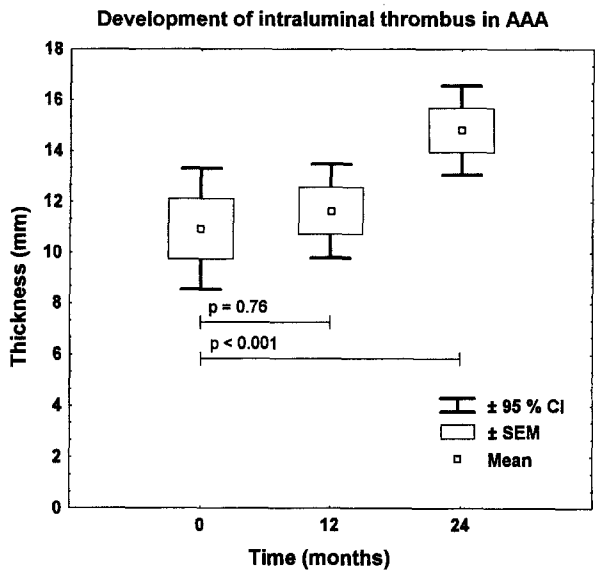

Fig. 2. Thickness of intraluminal thrombus in 55 patients with AAAs monitored for at least 24 months. $p < 0.001$. Results of post-hoc evaluation were calculated by Scheffe's method.

Table I. Correlations between initial diameter of AAA, thickness of thrombus, s-PIIINP, s-PICP, and age in 139 patients

	Diameter (cm)	*Thickness of thrombus (mm)*	*s-PIIINP (μg/L)*	*s-PICP (μg/L)*
Age (yr)	0.04 (0.02)	0.03 (0.73)	0.20 (0.02)	0.01 (0.95)
Diameter (cm)	—	0.48 (<0.001)	0.22 (0.01)	−0.01 (0.93)
Thickness of thrombus (mm)	—	—	0.49 (<0.001)	−0.09 (0.38)
s-PIIINP (μg/L)	—	—	—	0.14 (0.19)

Pearson product moment correlation coefficients are shown, with *p* values in parentheses.

tients who had a ruptured AAA were admitted to Oulu University Hospital between November 1992 and December 1995. The demographic data on the former group reflect typical findings of patients who have AAAs. The patients had a mean age of 70 years (range, 45 to 87 years), 111 of them were men (80%), 95 had coronary artery disease (69%), 90 had hypertension (65%), and 48 had chronic obstructive pulmonary disease (34%).

This asymptomatic group was followed-up at intervals of 6 to 12 months by means of a clinical examination, a B-mode ultrasound scan, and collection of samples for the identification of markers of collagen metabolism. The dimensions of the aneurysm and the intraluminal thrombus were recorded at each visit, and serum concentrations of the PIIINP and the PICP were measured, together with routine laboratory variables. None of the patients in the follow-up experienced a rupture of the AAA. In the patients whose AAA ruptured, blood was taken immediately on admission to minimize the time from the onset of symptoms. Fifty-five patients who had complete follow-up data at admission and at 12 and 24 months are presented separately in the results. Thirty patients had already been followed-up for 30 months, and their data are used to evaluate the speed of the changes at different times during the course of AAA disease. The patients who had a ruptured AAA are presented separately.

There were no patients in the series who had a history of any condition that is known to interfere with collagen metabolism (such as liver fibrosis), who had any malignancy, or who had undergone an operation less than a year before the first sampling.[9-11]

The concentrations of PIIINP[6] and PICP[7] were analyzed with equilibrium-type radioimmunoassays based on the human antigens (Orion Diagnostica, SF-90460, Oulunsalo, Finland) using duplicate

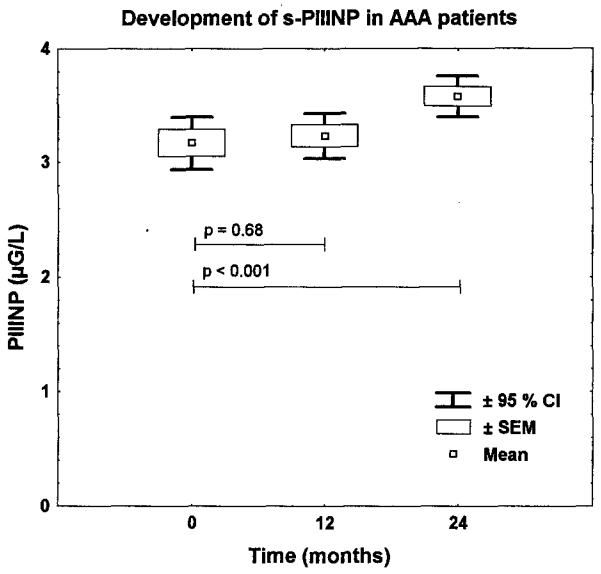

Fig. 3. Concentrations of PIIINP (aminoterminal propeptide of type III procollagen) in serum of 55 patients with AAAs monitored for at least 24 months. $p < 0.0001$. Results of post-hoc evaluation were calculated by Scheffe's method.

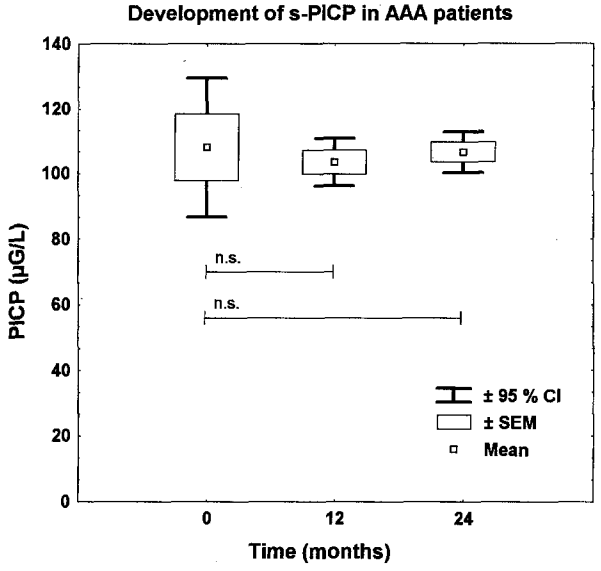

Fig. 4. Concentrations of PICP (carboxyterminal propeptide of type I procollagen) in serum of 55 patients with AAAs monitored for at least 24 months. $p = 0.9$. Results of post-hoc evaluation were calculated by Scheffe's method.

Table II. Rates of change in AAA diameter, thickness of thrombus, and s-PIIINP

	First year	*Second year*	*Further 6 months*
	mean (n) 95% CI	*mean (n) 95% CI*	*mean (n) 95% CI*
Change in AAA diameter (cm)	0.21 (55) −0.12 to 0.30	0.32 (55) 0.21 to 0.43	0.29 (30) 0.19 to 0.38
Change in thickness of thrombus (mm)	0.71 (55) −1.46 to 2.87	3.19 (55) 2.07 to 4.30	2.17 (30) 0.56 to 3.77
Change in s-PIIINP (µg/L)	0.06 (55) −0.08 to 0.20	0.35 (55) 0.24 to 0.46	0.21 (30) 0.11 to 0.33

200-µl aliquots of serum. Only one molecular form of PICP, that directly derived from the synthesis of type I procollagen, has been found. The reference interval for serum PICP (s-PICP) is 50 to 70 µg/L for women and 38 to 202 µg/L for men. The PIIINP assay detects the authentic aminoterminal propeptide and another, somewhat larger, related collagenous antigen, the proportion of which is stable at varying concentrations of PIIINP in the same individual. The assay used here does not detect the small antigen form possibly derived from the degradation of partially processed type III pN-collagen deposited on the surface of collagen fibers, or the PIIINP molecule itself. The reference interval for s-PIIINP is 1.7 to 4.2 µg/L for women and men. The intraassay coefficients of variation of the methods are about 3% and 5% for PICP and PIIINP, respectively. The laboratory procedures were conducted essentially as described in the original work.

Data storage and statistical analyses were performed with Statistica Software Rel.4.5 (StatSoft, Inc., Tulsa, Okla.). Parametric methods were used, indicating means, standard deviations, and 95% confidence limits (CI) of the means as appropriate. Analysis of variance for repeated measures was used to evaluate the overall changes in the serial data. Scheffe's method was used for post-hoc testing between the original and follow-up values. Correlations were evaluated using the Pearson product moment correlation coefficient.

RESULTS

The mean s-PIIINP in the 139 asymptomatic patients on the first visit after the initiation of this

Table III. Correlations between changes in s-PIIINP, diameter of AAA, and thickness of thrombus

	First year		*Second year*		*Further 6 months*	
	Change of diameter, r (n)	*Change of thrombus, r (n)*	*Change of diameter, r (n)*	*Change of thrombus, r (n)*	*Change of diameter, r (n)*	*Change of thrombus, r (n)*
Change in s-PIIINP (all AAAs)	0.15 (55) $p = 0.26$	0.29 (55) $p = 0.03$	0.37 (55) $p = 0.005$	0.24 (55) $p = 0.08$	0.55 (30) $p = 0.002$	0.42 (30) $p = 0.02$
Change in s-PIIINP (AAAs with initial thrombus)	0.18 (40) $p = 0.25$	0.26 (40) $p = 0.1$	0.31 (40) $p = 0.045$	0.22 (40) $p = 0.17$	0.67 (19) $p = 0.002$	0.33 (19) $p = 0.17$

Changes in all the patients in the follow-up (row 1), were compared with those in the patients with a thrombus in the AAA at the start of the study (row 2). Pearson product moment correlation coefficients are shown.

Table IV. Data on 18 patients with ruptured AAA on adminission

Sex	*Age (yr)*	*Duration of symptoms (hr)*	*s-PIIINP (μg/L)*	*s-PICP (μg/L)*	*CRP (g/L)*
Female	80	12	5.6	167	5
Male	83	6	5.9	176	5
Male	64	10	3.8	89	5
Male	73	24	4.6	122	5
Male	71	12	2.6	—	5
Male	76	10	4.3	145	5
Male	78	3	4.5	132	23
Male	70	6	2.4	79	5
Male	71	8	3.9	99	15
Male	66	2	3.8	88	5
Male	72	4	5.4	134	5
Male	62	12	4.8	99	5
Male	71	8	4.3	88	5
Male	74	24	4.5	113	10
Female	65	4	3.6	86	5
Male	66	10	3.3	98	5
Male	73	5	5.0	145	15
Male	77	12	4.4	121	5
Mean (SD)	71.8 (5.7)	9.6 (5.9)	4.3 (0.9)	117 (29.5)	7.4 (5.1)

CRP, C-reactive protein.

study was 3.5 μg/L (95% CI of the mean, 3.30 to 3.65), and the mean s-PICP was 111 μg/L (CI, 102 to 120). At the same time, the mean diameter of the aneurysm was 4.1 cm (CI, 4.0 to 4.3), and the mean thickness of the intraluminal thrombus 13 mm (CI, 11.6 to 14.9). The correlations between the initial values obtained for the diameter of the AAA, the thickness of the thrombus, s-PIIINP, and s-PICP are shown in Table I. There were 33 AAAs out of the 139 that had no initial thrombus, and when these were excluded from the analysis the correlations were as follows: s-PIIINP versus diameter, 0.18 ($p = 0.06$); s-PIIINP versus thrombus, 0.38 ($p < 0.001$) and diameter versus thrombus, 0.59 ($p < 0.001$).

So far 55 patients have been followed-up for at least 2 years, the interval between the visits being determined on a clinical basis as either 6 or 12 months. Thus the number of patients varies at different points in time, but complete follow-up data are available at 12 and 24 months. To eliminate the effect of natural individual variation on the mean values, only the time points for which we had complete data were used. The mean age of the follow-up group (68.9 years; 95% CI of the mean, 66.4 to 71.0) did not significantly differ from that of the whole group. A continuous increase in the diameter of the AAA seemed to take place during the 24 months of follow-up (Fig. 1), whereas the thickness of the thrombus was significantly increased only at 24 months (Fig. 2). s-PIIINP was similarly increased at 24 months (Fig. 3), but s-PICP did not vary significantly with time (Fig. 4).

The changes in the variables with time during the follow-up are evaluated in Table II. The mean speed of the changes in AAA diameter and s-PIIINP seem to have increased in the course of AAA disease, whereas the results concerning the change in thickness of the thrombus are more discrete. The respective correlations between the changes are shown in Table III. Initially there is no significant correlation between the changes in AAA diameter and s-PIIINP, but the correlation increases steadily with time. The same is true whether all the AAAs or only those with a thrombus already present at the start of the study are included. The relation between the changes in thrombus and s-PIIINP tends to be lower than that between diameter and s-PIIINP, except in the first year.

The data on the patients whose AAA ruptured are presented in Table IV. Their mean s-PIIINP, 4.3 μg/L, was higher than that of the asymptomatic group, 3.5 μg/L. In fact, only four s-PIIINP values (22%) were lower than the upper 95% confidence limit (3.65 μg/L) of the main group. The individual s-PICP values for the rupture group were quite evenly distributed relative to the respective CIs for the main group even though the mean for s-PICP was slightly elevated. The mean s-PIIINP for the patients who had a ruptured AAA was also markedly

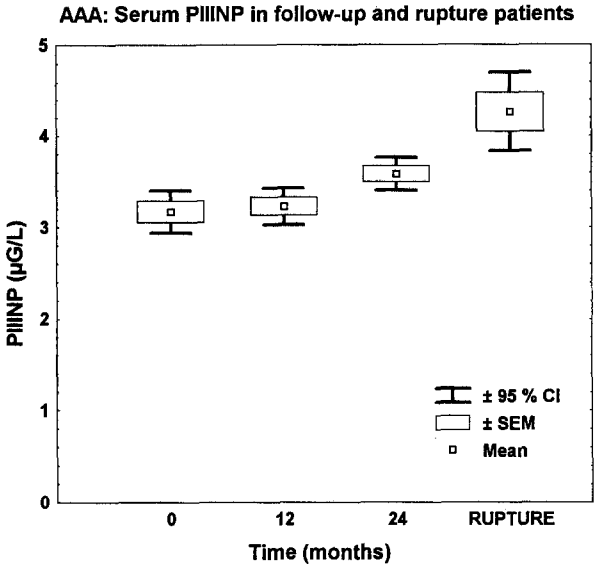

Fig. 5. Comparison of concentrations of PIIINP in serum of 55 patients with AAA during follow-up (0, 12, and 24 months) and patients who had a ruptured AAA on their first admission.

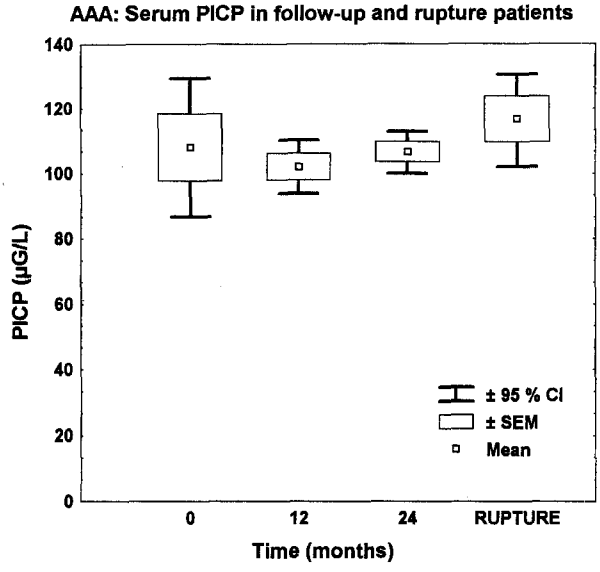

Fig. 6. Comparison of concentrations of PICP in serum of 55 patients with AAA during follow-up (0, 12, and 24 months) and patients who had a ruptured AAA already on first admission.

higher than that for the 55 follow-up patients at any point in time (Fig. 5). In fact, a tendency for elevated s-PICP was also seen in the same comparison, but the changes were not significant (Fig. 6). The correlation coefficient between s-PIIINP and s-PICP in the rupture group, 0.83 ($p < 0.001$), was much higher than that in the main group (Table I). Four patients (Table IV) showed a negligible increase in serum C reactive protein (CRP), with no exceptional respective increases in s-PIIINP or s-PICP. The samples were obtained less than 24 hours after the onset of symptoms in every case.

DISCUSSION

We have recently shown in a cross-sectional study that patients with AAAs have an increased turnover of type III collagen but not of type I collagen relative to patients who have femorodistal or aortoiliac occlusive arteriosclerotic disease. We were also able to identify the extra procollagen propeptide production as being at least in part derived from the aneurysmal sac.[8] The natural next question was whether further growth or rupture of the aneurysm might be reflected in the serum concentration of PIIINP.

The present results demonstrate a gradual increase in the diameter of the AAA, the thickness of the thrombus, and s-PIIINP during follow-up, whereas the change in s-PICP is negligible (Figs. 1 to 4). The changes in diameter and s-PIIINP tend to increase with time (Table II). The correlations between the changes in the main variables over the total duration of the follow-up were slight but distinct (data not shown), and it was after dividing the total study period into shorter intervals (Table III) that an increasing correlation emerged between the changes in AAA diameter and s-PIIINP with time, but this relationship was not as clear in the case of changes in the thickness of the intraluminal thrombus. Thus the accelerating growth of the aneurysm leads to an accelerating turnover of type III collagen in the wall of the aorta and is reflected in the total type III collagen metabolism, the net result being a rise in s-PIIINP.

The correlation between s-PIIINP and s-PICP varies between 0.26 in patients referred for hip surgery on account of arthrosis and 0.51 in patients awaiting elective major abdominal surgery (Haukipuro et al., unpublished data). The correlation found here, 0.14 (Table I), is unusually low and can only explained by abnormal metabolism of type III collagen in patients with AAA, as shown previously.[8] The type III collagen content of the aortic wall is high relative to many other tissues,[1] which causes the varying turnover in the aneurysm sac to be reflected in s-PIIINP. Conversely, the main reserve of type I collagen is in the skeletal system,[12] and exceptional synthesis of smooth tissue type I collagen (for example, in the healing of a surgical trauma[13]) is needed

before s-PICP values will increase, especially as s-PICP is not sensitive to the degradation of collagen.[7]

The original thickness of the thrombus per se showed an association with AAA diameter and s-PIIINP, just as our previous findings[8] pointed to a higher association between s-PIIINP and the size of the thrombus than between s-PIIINP and the diameter of the AAA. The thickness of the thrombus is a net effect of thrombogenesis and physiologic fibrinolysis, and this associated fibrinolysis could theoretically be a factor capable of increasing the degradation of collagen in the wall of the aorta through the effect of plasmin.[8,14] In fact, immunohistochemical studies have suggested that plasmin may be leached into the aortic wall from the mural thrombus.[15] It has also been hypothesized that a large thrombus may act as a barrier to the normal oxygen diffusion from the lumen to the inner layers of the aorta, thus leading to the natural course of AAA,[16] because it is a well-known fact that collagen synthesis is highly dependent on the presence of oxygen.[17] The thickness of the thrombus may also be connected with AAA rupture, as we found in our previous clinical series that there was a positive correlation between the maximum thickness of the thrombus and the tendency of the AAA to rupture.[18] This finding may suggest that the plasmin effect is more active in AAAs with larger clots and leads to increased collagenase activation, accelerated matrix destruction, more rapid AAA enlargement, and finally rupture.[18,19]

The s-PIIINP values were very high in the patients who had ruptured AAAs. Increased s-PIIINP can be found from the second day on after surgical trauma as a result of the reparative process as a part of the acute phase response.[10] Rupture of an AAA must evidently be a trauma capable of initiating a similar acute phase response. The lack of any measurable acute phase reaction, that is, low serum CRP values,[20] and the short intervals between the onset of symptoms and admission indicate that the cause of high s-PIIINP is not the acute phase response to the rupture itself but the accelerated turnover of type III collagen in the AAA toward the end point of the disease. The s-PICP values also tended to be high in the rupture group, and the correlation between s-PIIINP and s-PICP in particular, 0.83, is extremely high compared with other clinical situations, with the possible exception of those involving the formation of new tissue. These findings suggest that some degree of collagen synthesis must be present, because the peak in PICP could have been brought forward by the "background noise" caused by the whole-body type I collagen, the total mass of which is much greater than that of type III collagen. Thus this linear expression of PICP and PIIINP in cases of ruptured AAA may indicate a more active rate of both type I and type III collagen metabolism close to the rupture event.

CONCLUSION

Patients with AAAs have a abnormality in the metabolism of type III collagen, and further growth of the aneurysm is associated with accelerating turnover of type III collagen. The finding that patients who have aneurysm rupture on their first admission had abnormally high propeptide concentrations suggests that s-PIIINP may predict an approaching rupture event. However, this point needs to be studied further.

REFERENCES

1. Morton LF, Barnes MJ. Collagen polymorphism in the normal and diseased blood vessel wall: investigation of collagen types I, III, and V. Atherosclerosis 1982;42:41-51.
2. Dobrin PB, Baker WH, Gley WC. Elastolytic and collagenolytic studies of arteries: implications for the mechanical properties of aneurysms. Arch Surg 1984;119:405-9.
3. Sumner DS, Hokanson DE, Strandness DE Jr. Stress-strain characteristics and collagen-elastin content of abdominal aortic aneurysms. Surg Gynecol Obstet 1970;130:459-66.
4. Dubick MA, Hunter GC, Perez-Lizano E. Assessment of the role of pancreatic proteases in human abdominal aortic aneurysms and occlusive disease. Clin Chim Acta 1988;177:1-10.
5. Menashi S, Campa JS, Greenhalgh RM, Powell JT. Collagen in abdominal aortic aneurysm: typing, content, and degradation. J Vasc Surg 1987;6:578-82.
6. Risteli J, Niemi S, Triverdi P. Rapid equilibrium radioimmunoassay for the aminoterminal propeptide of human type III collagen. Clin Chem 1988;34:715-8.
7. Melkko J, Niemi S, Risteli L, Risteli J. Radioimmunoassay for the carboxyterminal propeptide of human type I procollagen. Clin Chem 1990;36:1328-32.
8. Satta J, Juvonen T, Haukipuro K, Juvonen M, Kairaluoma MI. Increased turnover of collagen in abdominal aortic aneurysms, demonstrated by measuring the concentration of the aminoterminal propeptide of type III procollagen in peripheral and aortal blood samples. J Vasc Surg 1995;22:155-60.
9. Kauppila A, Puistola U, Risteli J, Risteli L. Aminoterminal propeptide of type III procollagen: a new prognostic indicator in human ovarian cancer. Cancer Res 1989;49:1885-9.
10. Haukipuro K, Risteli L, Kairaluoma MI, Risteli J. Aminoterminal propeptide of type III procollagen in serum during wound healing in human beings. Surgery 1990;107:381-8.
11. Wiklund TA, Elomaa I, Blomqvist CP, Risteli L, Risteli J. Type III collagen metabolism in soft tissue sarcomas. Br J Cancer 1992;65:193-6.
12. Risteli J, Risteli L. Markers of collagen assembly and turnover. In: Lindh E, Thorell JI, editors. Clinical impact of bone and connective tissue markers. New York: Academic Press Inc., 1989:201-9.
13. Haukipuro K, Melkko J, Risteli L, Kairaluoma MI, Risteli J. Connective tissue response to major surgery and postoperative infection. Eur J Clin Invest 1992;22:333-40.

14. Peuhkurinen KJ, Risteli L, Melkko JT, Linnaluoto M, Jounela A, Risteli J. Thrombolytic therapy with streptokinase stimulates collagen breakdown. Circulation 1991;83:1969-75.
15. Jean-Claude J, Newman KM, Hong L, Gregory AK, Tilson MD. Possible key role for plasmin in the pathogenesis of abdominal aortic aneurysms. Surgery 1994;116:472-8.
16. Vorp DA, Federspiel WJ, Webster MW. Does laminated intraluminal thrombus within abdominal aortic aneurysm cause anoxia of the aortic wall [letter]? J Vasc Surg 1996;23:540-1.
17. Prockop DJ, Kivirikko KI, Tuderman L, Guzman NA. The biosynthesis of collagen and its disorders. N Engl J Med 1979;301:77-85.
18. Satta J, Läärä E, Juvonen T. Intraluminal thrombus predicts rupture of an abdominal aortic aneurysm [letter]. J Vasc Surg 1996;23:737-9.
19. Wolf YG, Thomas WS, Brennan FJ, Goff WG, Sise MJ, Bernstein EF. Computed tomography scanning findings associated with rapid expansion of abdominal aortic aneurysm. J Vasc Surg 1994;20:529-38.
20. Colley CM, Fleck A, Goode AW, Muller BR, Myers MA. Early time course of the acute phase protein response in man. J Clin Pathol 1983;36:203-7.

Submitted July 19, 1996; accepted Oct. 21, 1996.